Association of Aphanomyces cladogamus with severe root rot of pansies

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With plate XI.—XVI.

Buisman (1927), working in the Netherlands, was the first investigator to mention having isolated an *Aphanomyces* from decaying roots of pansies (***Viola tricolor*** L.). The fungus she obtained looked to her very similar to my *A. euteiches* (Jones and Drechsler, 1925), but in her inoculation experiments it did not attack pea (***Pisum sativum*** L.) roots. From lack of time she was unable to ascertain experimentally whether her *Aphanomyces* was the cause of an acute root rot affecting pansies in her experimental garden. Soon afterwards Meurs (1928), also working in the Netherlands, carried out inoculation experiments which showed that the *Aphanomyces* from pansy roots was capable of attacking roots of both *V. tricolor* and *V. cornuta* L. He concluded accordingly that the fungus was the cause of an acute root rot of pansies in Holland. In supplementary inoculation experiments he determined further that *A. euteiches* from diseased pea roots would not infect either *V. tricolor* or *V. cornuta*. As Meurs, like Buisman, considered the pansy fungus to be morphologically identical with *A. euteiches* despite some differences in growth and parasitism he designated it as *A. euteiches* P. F. 2. The same designation he applied likewise to cultures of *Aphanomyces* isolated by him from roots of spinach (*Spinacia oleracea* L.) and *Arabis alpina* L.

The association of an *Aphanomyces* with severe disease in Violas came to my attention more than 20 years ago (Drechsler, 1934), when a generous collection of affected pansies was received for determination of whatever injurious fungi might be present. These diseased specimens were taken on April 29, 1933, from a garden in Mount Rainier, Maryland, in which pansies had been severely affected with root rot for several years. Their leaves, though well developed and with little discoloration, showed wilting in noticeable degree. Their stems appeared of normal color and undiminished firmness. Their luxuriant root systems, after being washed free of soil, were also found of normal firmness, and the root epidermis everywhere appeared free of any discoloration or external blemish. Yet when the root systems were viewed under bright illumination against a white background each showed an extensively and conspicuously darkened core. On removal of the outer cortical sheath the vascular cylinder was exposed to view as a deep red or orange-red strand. Mounts prepared from thin slices or thin fragments of affected roots showed the parenchyma tissue to be virtually undamaged and without any general invasion by bacteria. Along and within the central cylinder, however, numerous oospores were found distributed rather uniformly, though here also, bacteria, the usual escort of phycomycetous invaders, were for the most part not discernible. Many of the oospores, being wedged in the unyielding fabric of the stele, were found constrained into various elongated ellipsoidal shapes. Regardless of modifications in outward form all oospores that had reached a mature resting condition showed in the parietal protoplasmic layer surrounding the central reserve globule the geometrical arrangement of largish sphaeroidal granules familiar in the oospores of many saprolegniaceous fungi. The thorough-going distribution of the oospores, and the frequent absence of any other visible microorganism in the roots, gave very convincing reason for considering the oomycete present as the agent responsible for the wilting of the leaves. When portions of diseased root were planted on Petri plates of maize-meal agar mycelia of an *Aphanomyces* grew out promptly and consistently. No other organism of a likely pathogenic character appeared in the resulting cultures.

The garden from which the incipiently wilting specimens were taken still showed on inspection 20 days later (on May 19, 1933) many pansy plants of erect posture and generally healthy aspect (Pl. XI, A, left, right; B, right). At distances of only 20 or 30 centimeters from some of these healthy individuals neighboring plants were found wilted down beyond recovery (Pl. XI, A, middle) though the leveled foliage and stems in some instances still seemed alive at the time. Often, again, the wilting in closely neighboring plants was found more advanced, with the prostrated stems, leaves, and flowers drying out and shrivelling (Pl. XI, B, middle). In some borders a row of 3 or 4 badly withered plants (Pl. XII, A, center) were found flanked by plants that showed only incipient wilting (Pl. XII, A, lower left, upper right). The final stage of destruction came to light in instances where denuded stems and peduncles lay extended on the ground intermingled with shrivelled remains of leaves and flowers (Pl. XII, B, lower middle).

In the more severely affected pansy plants the basal portion of the stem always showed pronounced injury from softening, discoloration or decay of its cortex. At the ground line little frequently would be left of the stem except the denuded core. Above the denuded portion the cortical layer of the stem was often softened for a distance of 5 to 25 mm. The cortical layer of the roots below the denuded portion of the stem likewise showed more or less extensive softening and discoloration. Owing to the conspicuous injury they displayed externally, plants in the later stages of attack had little the appearance of succumbing to internal infection along the woody stele. When portions of softened stem or root were now bathed in sterile water for 5 to 15 hours, and after being pressed between sheets of filter paper were transferred to Petri plates of maize-meal agar, mycelia soon grew out that yielded pure cultures not only of an *Aphanomyces* but also of several species of *Pythium*.

Since 1933 pansy plants with severe root rot have been received repeatedlyfrom home gardens in and near Washington, D. C. As the specimens would seem usually to have been gathered in late stages of decline it was not possible, as a rule, to determine whether attack had been initiated with invasion by an *Aphanomyces* along the central stele. Most often the plants were broken off near the ground level, having apparently been pulled up without much care. Nevertheless, by cutting pieces of affected stem tissue from these ineptly collected specimens, and bathing them in sterile water for several hours before placing them on maize-meal agar plates, it was usually possible to obtain pure cultures of the familiar *Aphanomyces* as well as of several species of *Pythium*.

Despite the habitual recurrence of severe root rot in some gardens, especially in years of liberal spring rainfall, this disease would yet seem wholly absent in many pansy beds in and near Washington, D. C. The rather extensive borders on the grounds of the Plant Industry Station, near Beltsville, Maryland, that have been planted with pansies for more than 15 years in succession, have so far shown only a few examples of severe root rot. The seedlings set out in these borders were always grown in unsterilized soil except in one year when sterilized soil was employed incidentally. In some wet years when the pansies in these borders were dug up after the middle of May to be replaced by other ornamentals better adapted to the hot summers of the region, examination of their well-developed root systems revealed only very meager injury in that the epidermis here and there was darkened or discolored in rather vaguely delimited areas. Such discoloration is not uncommon on pansy roots in and near Washington, D. C., often appearing to some extent even when only moderate rainfall has come early in spring. It has no evident association with wilting or discoloration of leaves and stem, and is not known to usher in the severe type of root decay. From the superficially discolored portions of root numerous isolations of *Pythium ultimum* Trow, *P. debaryanum* Hesse, *P. mamillatum* Meurs, *P. vexans*

De Bary, *P. oligandrum* Drechsl., and *P. acanthicum* Drechsl. have been obtained, but no isolations referable to any species of *Aphanomyces*. Nor were any *Aphanomyces* cultures obtained from the softened stems and roots of the pansy specimens originating from Washington, D. C., in 1938 (Drechsler, 1949) that yielded isolations of the distinctive *P. violae* Chesters and Hickman (1944).

Although the gardens from which came the diseased pansies that yielded *Aphanomyces* cultures varied moderately with respect to texture and fertility of the soil, all the *Aphanomyces* cultures obtained from pansies in the Washington area during 20 years resembled one another closely in growth habit and morphology. The figures (Pl. XIII, A—H; Pl. XIV, A—H) showing 16 units of sexual reproductive apparatus formed in a culture isolated from a diseased pansy taken from a home garden in Arlington, Virginia, late in May, 1939, illustrate tolerably the more usual variations in make-up characteristic also of many other isolations. Thus a generally satisfactory agreement in make-up is evident when the 16 reproductive units are compared with homologous units (Pl. XV, A—G) formed in a culture isolated from a pansy plant found succumbing to root rot in a garden near Bethesda, Maryland, on May 23, 1953. The assortment of sexual apparatus produced by the isolation obtained from Virginia and the assortment produced by the isolation originating 14 years later from a locality 10 kilometers away both reveal obvious parallelism with the 13 sexual reproductive units earlier presented in figures (Drechsler 1929, p. 330, 331) illustrating the tomato-root fungus on which was based the original description of *A. cladogamus*.

In the pansy fungus, as in the tomato-root fungus, the hypha (Pl. XIII, A—C: a; Pl. XIV, A—D: a; Pl. XV, A, a) bearing the oogonial stalk often has no close or visible connection with the hypha (Pl. XIII, A—C: b; Pl. XIV, A—D: b; Pl. XV, A, b) bearing the antheridial branches. Yet very often, again, the oogonial stalk (Pl. XIII, D—H: a; Pl. XIV, E—F: a; Pl. XV, B—G: a) originates from the same hypha as the antheridial branch (Pl. XIII, D—H: b; Pl. XIV, E—F: b; Pl. XV, B—G: b) the resulting monoclinous relationship being, indeed, a feature especially characteristic of the species. Sometimes the hypha that gives off the oogonial stalk (Pl. XIV, G, a; H, a) gives off two antheridial branches each of which may supply one (Pl. XIV, H, b, c) or more (Pl. XIV, G, b, c) male cells. In rather compact monoclinous reproductive units the total length of the hyphal elements connecting oogonium and antheridium may scarcely exceed 100 or 125 μ (Pl. XIII, G; Pl. XIV, F; Pl. XV, F) while in rangier monoclinous units the oogonial stalk, antheridial branch, and connecting portion of parent hypha may have an aggregate length of approximately 250 μ (Pl. XIV, G, a, c; H, a, c; Pl. XV, C, a, b). Short diverticulations and spurs are often borne here and there on the sexual branches so that even reproductive units with only one or two antheridia sometimes will present a fairly intricate appearance. The generally bulbous shape of the antheridium is often modified, much as in the tomato-root fungus, by the presence of a lateral hyphal appendage or of a distal prolongation. The oogonial stalk in diclinous (Pl. XIII, A, C; Pl. XIV, D; Pl. XV, A) as also in monoclinous (Pl. XIII, D; Pl. XIV, E; Pl. XV, D) units sometimes winds spirally around an antheridial branch, or, again, an antheridial branch may wind noticeably around the oogonial stalk (Pl. XIII, G; Pl. XIV, F), but in comparison with the extensive enwrapment of oogonia by antheridial branches the entwinement of one sexual branch by the other is not usually a conspicuous feature in the development of reproductive apparatus under the surface of agar substratum.

Two hundred oogonia selected at random in maize-meal agar plate cultures of the Bethesda isolation gave measurements for diameter, expressed in the nearest integral number of microns, with a distribution as follow: 21 μ, 2; 22 μ, 9; 23 μ, 13; 24 μ, 27; 25 μ, 46; 26 μ, 32; 27 μ, 29; 28 μ, 19; 29 μ, 11; 30 μ, 8; 31 μ, 3; 35 μ, 1; and the mature oospores contained in them — all of correct internal organization — gave measurements for diameter distributed thus: 17 μ, 2; 18 μ, 6; 19 μ, 19; 20 μ, 41; 21 μ, 61; 22 μ, 43; 23 μ, 18;35 μ, 1; and the From the two sets of measurements averages of 25.8 μ and 21.0 μ were computed for oogonial diameter and oospore diameter, respectively. Varying from 0.5 to 1.2 μ, the supplementary measurements for thickness of oogonial envelope averaged 0.8 μ, while the measurements for thickness of oospore diameter, with a range from 1.2 to 2.1 μ, gave an average of 1.6 μ. Measurements for diameter of the reserve globule here ranged from 8.2 to 13.5 μ and averaged 10 μ. The main dimensions of oogonium and oospore in the pansy fungus thus would appear to agree satisfactorily with the corresponding dimensions in isolations of *Aphanomyces cladogamus* from roots of tomatoes and of spinach and flax (Drechsler, 1954).

When portions of natural or artificial substratum well permeated with mycelium of the pansy *Aphanomyces* are shallowly immersed in distilled water the fungus extends numerous hyphae into the surrounding liquid. Many of these hyphae later serve as evacuation tubes in the discharge of extensively ramified sporangial units. Often the evacuation hyphae become modified for dehiscence not only at the axial tip (Pl. XVI, A, a; B, a) but also at the tips of one or more short branches or spurs (Pl. XVI, A, b, c; B, b) borne laterally in distal positions, though not all of the available tips need actually operate in discharging the zoospores. In instances where the axial tip and a single lateral spur would seem to offer equally favorable passageways the axial tip (Pl. XVI, C, a) sometimes remains closed, with the result that all the zoospores escape through the open spur (Pl. XVI, C, b), whereas at other times the lateral spur remains closed (Pl. XVI, D, b) and all the zoospores are released from the open axial tip (Pl. XVI, D, a). Evacuation tubes with five (Pl. XVI, E, a—e) or six (Pl. XVI, F, a—f) available tips may liberate zoospores from several openings (Pl. XVI, E, a, b, c, e; F, a, b, d, e) even should one (Pl. XVI, E, d) or more (Pl. XVI, F, c, f) of the tips remain closed. By far the largest number of zoospores are usually released from the tip that happens to open first, for discharge ordinarily proceeds most rapidly in the earliest stages, when perhaps 10 to 15 naked protoplasts are violently expelled each second. After rounding and encysting in haphazard disorder near the hyphal aperture from which they emerged the individual zoospores (Pl. XVI, G, a—z; I, a—p) mostly measure 7 to 8 μ in diameter. A few scattered oversized specimens as much as 13 or 14 μ in diameter (Pl. XVI, H, a, b), which presumably derive from imperfect cleavage within the sporangium, are usually present in all irrigated material. If their germinition is long delayed the encysted zoospores, whether of ordinary (Pl. XVI, J, a—d) or of more than ordinary (Pl. XVI, J, e, f) size, often become rather strongly vacuolated and noticeably larger. Zoospores germinating soon after their encystment, and in water almost devoid of nutrients, commonly extend a germ hypha only 2 to 2.5 μ wide (Pl. XVI, K—O). Though yielding encysted zoospores in enormous numbers all the *Aphanomyces* cultures I have isolated from pansies have been reluctant to produce motile zoospores. No motile zoospores whatever came under observation in the irrigated material of the Bethesda isolation used in making the drawings of asexual reproductive apparatus shown in Pl. XVI. Pronounced reluctance of *A. cladogamus* to form motile zoospores under conditions highly suitable for development of the motile stage in congeneric root-rotting parasites was noted in my description of the species (Drechsler, 1929), and more recently was observed likewise by McKeen (1952) in isolations he obtained from pepper (*Capsicum frutescens* L.) seedlings that had damped-off under glass.

The pansy *Aphanomyces* readily infects seedlings of the three vegetable crop plants — tomato, pepper, and spinach — on which *A. cladogamus* has been found to occur spontaneously. Seed of the three vegetables was sown in sterilized dishes filled with sterilized sand through which had been distributed small pieces of maize-meal agar well permeated with vigorous mycelium of the Bethesda isolation, each 100 square centimeters having received an admixture of about 1 gram of permeated agar. Distilled water free of alien oomycetes was used to keep the sand continuously moist. Approximately one-third of the tomato seedlings that emerged succumbed to damping-off. Fully three-fourths of all pepper seedlings damped-off either before or after emergence. Less than one-tenth of all spinach seedlings escaped destruction. When infected seedlings were placed on maize-meal agar plates they always yielded *Aphanomyces* mycelium very promptly. In the tests spinach showed generally such pronounced liability to attack by the pansy *Aphanomyces* as to suggest that the fungus may at times cause serious damage to young stands of this vegetable in garden or field. Eggplant (*Solanum melongena* L.) seedlings were included in the inoculation trials, and were found to succumb to invasion by the pansy *Aphanomyces* in about the same measure as pepper seedlings.

In view of very close agreement with respect to biological relationships as well as with respect to morphology of both sexual and asexual reproductive apparatus the *Aphanomyces* found associated with severe root rot of pansies in and near the District of Columbia is held to be identical with *A. cladogamus.*

References.

Buisman, C. J. Root rots caused by phycomycetes. Meded. Phytopath. Labor. Willie Commelin Scholten **11**: 1—51. 1927.

Chesters, C. G. S. and Hickman, C. J. On *Pythium violae* n. sp. and *P. oligandrum* Drechsler from cultivated *Viola*. Tran. Brit. Mycol. Soc. **27**: 55—62. 1944.

Drechsler, C. The beet water mold and several related root parasites. Jour. Agr. Research **38**: 309—361. 1929.

— Vascular wilt and root rot of pansies due to *Aphanomyces* sp. (Abstract) Phytopathology **24**: 7—8. 1934.

— Longevity of some *Pythium* species in maize-meal agar cultures. (Abstract) Phytopathology **39**: 503. 1949.

— Morphological features shown in *Aphanomyces* isolations from roots of spinach and flax. Jour. Washington Acad. Sci. **44**: 212—219, 1954.

Jones, F. R. and Drechsler, C. Root rot of peas in the United States caused by *Aphanomyces euteiches* (n. sp.). Jour. Agr. Research **30**: 293—325. 1925.

McKeen, C. D. *Aphanomyces cladogamus* Drech., a cause of damping-off in peppers and certain other vegetables. Canad. Jour. Bot. **30**: 701—709, pl. 1—3. 1952.

Meurs, A. Wortelrot, veroorzaakt door schimmels uit de geslacten *Pythium* Pringsheim en *Aphanomyces* De Bary. 95 pp., Baarn. 1928.

Explanation of plates XI—XVI

Plate XI. Pansy plants (among other ornamentals) photographed on May 19, 1933, in a garden in Mount Rainier, Maryland, from which had been obtained 20 days earlier many pansy specimens with extensive red stele infection of their root systems. A, Badly wilted pansy (near center) between apparently healthy specimens on left and right. B, Severely withered pansy (left) near an apparently healthy one (right).

Plate XII. Pansy plants (among other ornamentals) photographed on May 19, 1933, in a garden in Mount Rainier, Maryland, from which had been obtained 20 days earlier many pansy specimens with extensive red stele infection of their root systems. A, Severely withered pansies (near center) between pansy specimens (lower left and upper right) showing only incipient wilting. B, Dead remains of a pansy (lower right) that succumbed to severe root rot at a distance only about 50 cm. from a plant (left) apparently still healthy.

Plate XIII. Sexual reproductive apparatus of an isolation of *Aphanomyces cladogamus* obtained in May, 1939, from a decaying root of a diseased pansy taken from a garden in Arlington, Virginia; all reproductive units produced on the under side of maize-meal agar plate cultures, and drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A—C, Diclinous reproductive units with one mycelial hypha (a) giving off the oogonial stalk, and another hypha (b) supplying the branches bearing the plural antheridia; oospore immature in A, mature in B and C. D—H, Mature monoclinous reproductive units, showing close mycelial connection between the hyphal branch (a) supplying the oogonium and the hyphal branch (b) supplying the male complement consisting of one (G) or more (D, E, F, H) antheridia.

Plate XIV. Sexual reproductive apparatus of an isolation of *Aphanomyces cladogamus* obtained in May, 1939, from a decaying root of a diseased pansy taken from a garden in Arlington, Virginia; all reproductive units formed on the under side of maizemeal agar plate cultures, and drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A—D, Diclinous reproductive units, with one mycelial hypha (a) supplying the oogonium, and another hypha (b) supplying the male complement consisting of one (C, D) or more (A, B) antheridia. E, F, Monoclinous reproductive units showing close mycelial connection between the hyphal branch (a) supplying the oogonium and the hyphal branch (b) supplying the male complement consisting of one (F) or more (E) antheridia. G, H, Monoclinous reproductive units in each of which the hyphal branch (a) supplying the oogonium is given off from the same mycelial filament from which arise also the 2 branches, b—c, that together supply the attendant antheridia. Oospore slightly immature in E and H; fully mature in A, B, C, D, F.

Plate XV. Sexual reproductive apparatus of an isolation of *Aphanomyces cladogamus* obtained from decaying stem tissue of a collapsing pansy taken from a garden near Bethesda, Maryland, on May 23, 1953; all reproductive units formed on the under side of maize-meal agar plate cultures, and drawn to a uniform magnification with the aid of a camera lucida; $\times 1000$ throughout. A, Diclinous reproductive unit, with one mycelial hypha (a) supplying the oogonium, and another hypha (b) supplying the two attendant antheridia. B—G, Monoclinous reproductive units in each of which the same mycelial filament gives off both the hyphal branch (a) supplying the oogonium and the hyphal branch (b) supplying the one (C, D, E) or two (B, F, G) attendant antheridia. Oospore slightly immature in B and C, but in fully mature resting condition in A, D, E, F, G.

Plate XVI. Asexual reproductive apparatus of the same isolation of *Aphanomyces cladogamus* as is shown in Plate XV; all parts formed in irrigated Lima-bean agar preparations and drawn with the aid of a camera lucida. A, Terminal portion of evacuation tube about 15 minutes before sporangial discharge, showing three tips (a—c) available for dehiscence; $\times 500$. B, Terminal portion of evacuation tube about 2 minutes before sporangial discharge, showing two tips (a, b) available for dehiscence; $\times 500$. C, Same after discharge of sporangium, showing axial tip (a) closed and lateral tip (b) open; $\times 500$. D, Distal portion of another evacuation tube after discharge of sporangium, showing axial tip (a) open and lateral tip (b) closed; $\times 500$. E, Distal portion of evacuation tube in late stage of sporangial discharge, showing four open (a—c, e) and one closed (d) tip; $\times 500$. F, Distal portion of evacuation tube in late stage of sporangial discharge, showing four open (a, b, d, e) and two closed (c, f) tips; $\times 500$. G, Newly encysted zoospores (a—z) of usual size; $\times 500$. H, Newly encysted oversized zoospores (a, b) resulting from imperfect cleavage in sporangium; $\times 500$. I, Newly encysted zoospores (a—p) of usual size; $\times 1000$. J, Zoospores of usual (a—d) and of larger than usual (e, f) sizes, showing pronounced vacuolization and enlargement two days after their encystment; $\times 1000$. K—O, Zoospores germinating in water; $\times 500$. (Owing to lack of space E and F are shown in parts whose proper connection is indicated by broken lines.)


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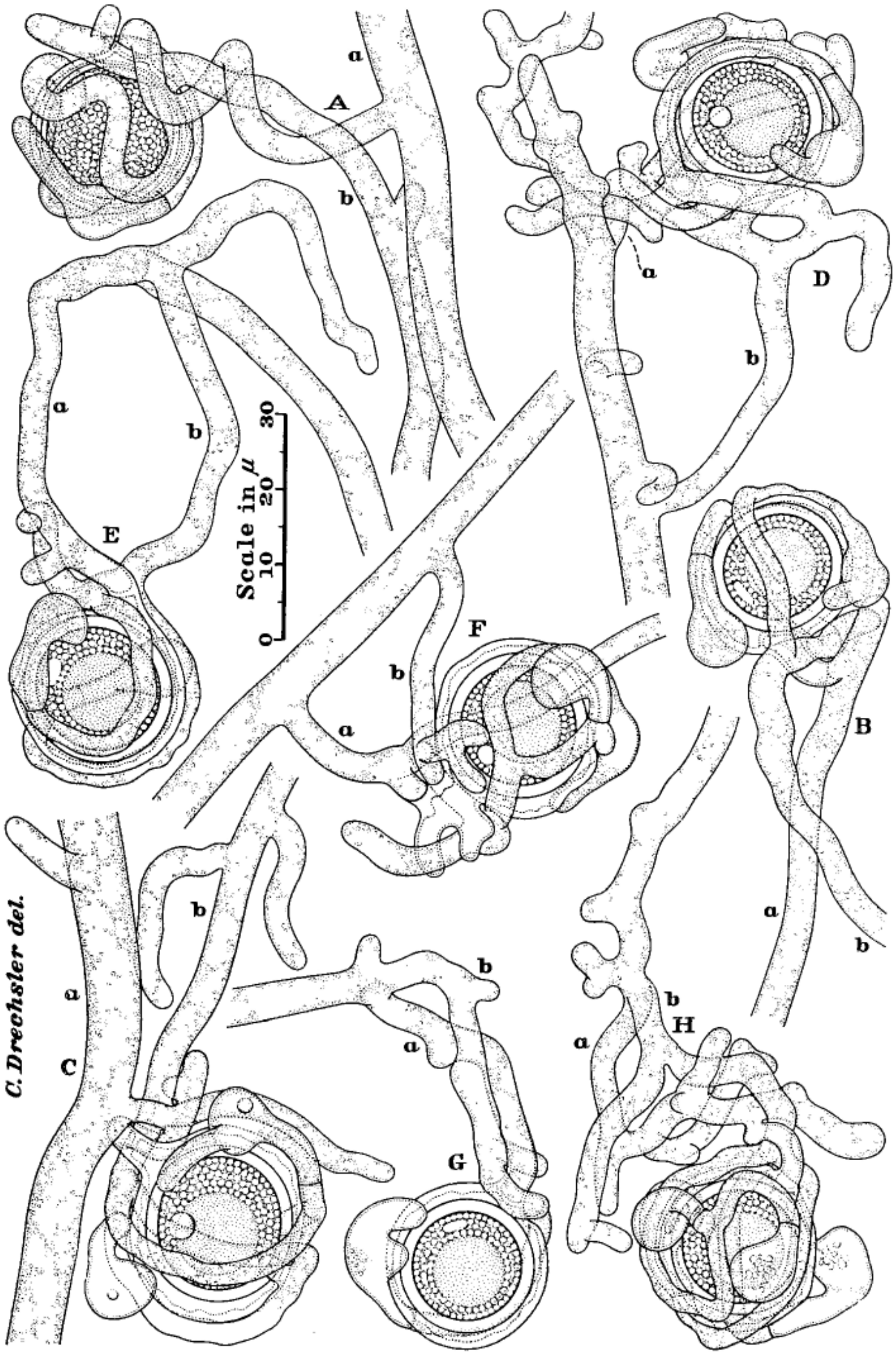
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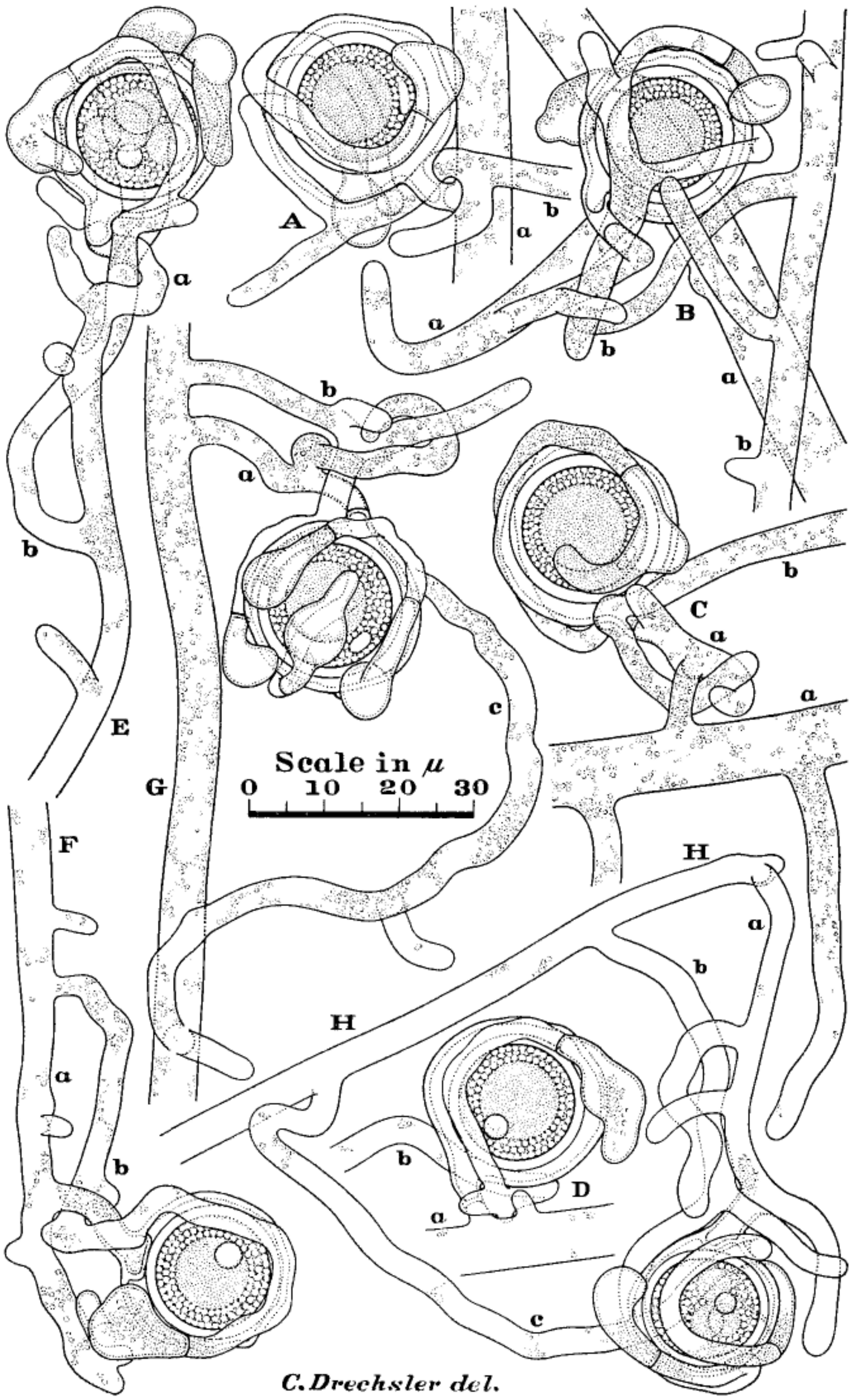

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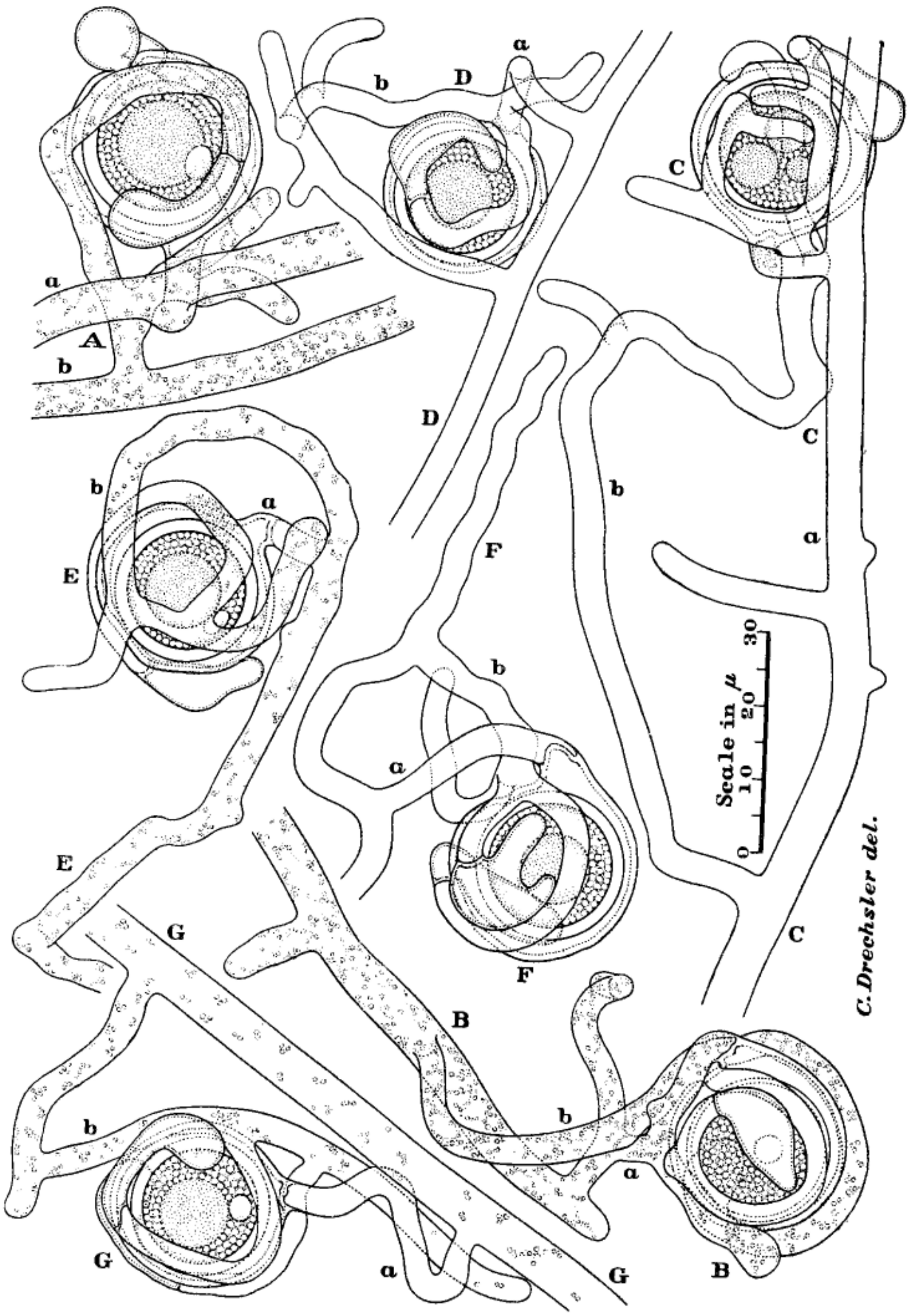
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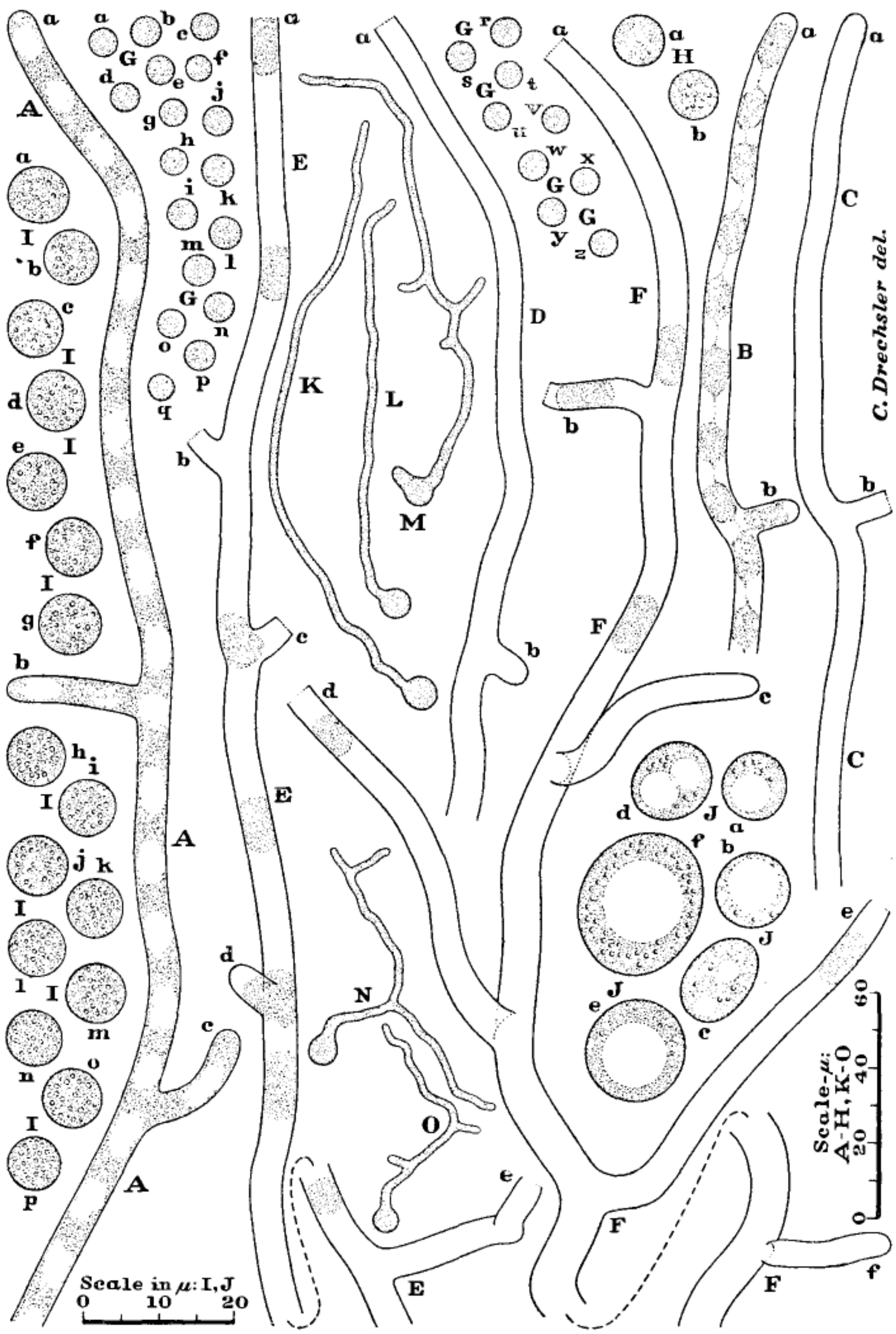
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